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Hyperthermia During Intraperitoneal Chemotherapy With Paclitaxel or Docetaxel for Ovarian Cancer: Is There Any Benefit?

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Abstract. *Background/aim: Intraperitoneal chemotherapy with taxanes provides high locoregional drug concentrations. Regarding their synergy with hyperthermia, results have been inconclusive. In this in vitro study, the thermal enhancement of the effect of paclitaxel and docetaxel on ovarian cancer cells under conditions mimicking those during hyperthermic intraperitoneal chemotherapy (HIPEC) is evaluated. Materials and Methods: Cisplatin-resistant SKOV-3 and OVCAR-3 ovarian cancer cells were exposed for 2 h to 0.1, 1 and 3 μM of paclitaxel and docetaxel at 37˚C (normothermia) and 41.5˚C (hyperthermia). Cell proliferation and cell-cycle distribution were evaluated after 24 h, 3 days and 7 days. Results: A concentration-dependent cytotoxic effect on cell proliferation was observed. Concurrent hyperthermia caused an increased arrest of cells in the G_2/M phase. At 7 days, thermal enhancement of drug effect was shown only for treatment of OVCAR-3 cells with 1 μM paclitaxel. Conclusion: The concentration-dependent cytotoxic effect of paclitaxel and docetaxel supports their intraperitoneal use. Due to the lack of or only minimal thermal enhancement, normothermic may be as effective as hyperthermic intraoperative intraperitoneal chemotherapy with taxanes, avoiding, however, potential oncological and treatment-related adverse effects of concurrent hyperthermia.*

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After cytoreductive surgery (CRS) for advanced ovarian cancer, inevitably at least residual microscopic peritoneal disease is left behind, which remains to be eliminated by chemotherapy. The taxanes paclitaxel and docetaxel are effective agents in the treatment of ovarian cancer by binding to and promoting the assembly of tubulin into dysfunctional microtubules, resulting in inhibition of mitosis and cell proliferation, and consequently the death of rapidly proliferating tumor cells (1, 2). The degree of exposure to paclitaxel and docetaxel is known to correlate with treatment response (1, 2). Intraperitoneal chemotherapy for residual peritoneal disease provides an evident pharmacokinetic advantage over intravenous chemotherapy, with high locoregional and simultaneously low systemic drug exposure. Paclitaxel and docetaxel have a highly favorable pharmacokinetic profile after intraperitoneal administration, mainly due to their high molecular weight and hydrophobic properties which delay absorption from the abdominal cavity (3, 4). Intraperitoneal chemotherapy with taxanes have been demonstrated to be effective in advanced ovarian cancer (5, 6). A prerequisite for effective intraperitoneal chemotherapy is optimal CRS, leaving no or only very small peritoneal metastases behind, since the penetration depth of intraperitoneally delivered drugs is limited. The intraoperative application of intraperitoneal chemotherapy directly after CRS prohibits inadequate exposure of the entire seroperitoneal surface to the drug solution due to formation of adhesions and allows early treatment before tumor regrowth occurs (7). CRS and hyperthermic intraoperative intraperitoneal chemotherapy (HIPEC) have been increasingly used in the treatment of primary and secondary peritoneal malignancies, including advanced ovarian cancer (8, 9).

The rationale for performing intraoperative intraperitoneal chemotherapy under hyperthermic conditions is that hyperthermia itself is considered cytotoxic and it enhances the cytotoxicity, as well as the tissue penetration, of many chemotherapeutic drugs (4, 7, 10-12). The target intra-abdominal temperature is usually between 40 and 42˚C for 30

to 120 minutes, according to the center's protocol. Local temperatures of 43˚C and higher may cause thermal injury to organs and other tissues (13). Although for platinum compounds thermal enhancement has been evidently demonstrated in the past (11, 12, 14-16), some recent *in vitro* studies on various gastrointestinal and ovarian cancer cell lines did not show such an effect at clinically relevant elevated temperatures (17, 18). Regarding the thermal enhancement of the cytotoxicity of paclitaxel and docetaxel, results of *in vitro* and *in vivo* studies have been inconclusive (3, 4, 12).

The present study examined whether *in vitro* cytotoxicity of paclitaxel and docetaxel to ovarian cancer cells is increased at higher drug concentrations, supporting their use for intraperitoneal chemotherapy. Moreover, whether the addition of hyperthermia, as in HIPEC, actually increases their cytotoxicity in ovarian cancer cell lines was also investigated. Lack of increase of cytotoxicity may support the use of intraoperative perfusion chemotherapy under normothermic conditions, avoiding the potential adverse effects of hyperthermia, which will be discussed further on.

Materials and Methods

Cell lines and culture. Cisplatin-resistant SKOV-3 and OVCAR-3 ovarian cancer cell lines, obtained from the American Type Tissue Culture Collection (Manassas, VA, USA), were maintained at 37˚C in a humidified atmosphere containing 5% CO_2. SKOV-3 cells were cultured in McCoy's 5A modified medium supplemented with 10% heat-inactivated fetal bovine serum and OVCAR-3 cells in RPMI1640 medium without phenol red, supplemented with 20% heat-inactivated fetal bovine serum, 50 IU ml^{-1} penicillin and 50 μg ml^{-1} streptomycin. All media were purchased from Biochrom (Berlin, Germany).

Cell treatments. Upon reaching 50-60% confluence, cells grown on culture dishes were incubated with 0.1, 1 or 3 μM paclitaxel (Taxol®; Bristol-Meyer Squibb, New York, NY, USA) or docetaxel (Taxotere®; Sanofi-Aventis, Paris, France) diluted in culture medium, at 37˚C (normothermia) or 41.5˚C (hyperthermia) for 2 h in a CO_2 incubator. Cells were then washed with fresh medium and incubated in drug-free medium at 37˚C for up to 7 days. Cells cultured without drugs at 37˚C were used as controls.

In our experimental protocol, micromolar rather than nanomolar concentrations were chosen to mimic conditions found during intraperitoneal chemotherapy (19, 20). Treatment of cells at a temperature of 41.5˚C for 2 h mimics the hyperthermic conditions under HIPEC according to the protocol used at our center.

Cell proliferation assays. Cells were grown on 24-well plates and after the various treatments cell proliferation was determined using 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), as specified by the supplier (Sigma Chemical Co, St Louis, MO, USA). The percentages of viable cells were determined by measurement of MTT absorbance and results were expressed as the percentage of viable cells by comparison to the maximal (100%) cell proliferation of untreated cultures (no paclitaxel or docetaxel, 37˚C). Measurements were performed 1, 3 and 7 days after each treatment. All cell proliferation data are the means of nine replicates from three independent experiments.

Cell-cycle analysis. Total, adherent and non-adherent, cells following drug, hyperthermia or physiological culture conditions were analyzed. In brief, adherent cells obtained after trypsinization and centrifugation were combined with non-adherent cells contained in the culture supernatant and the total cell population was washed with culture medium and phosphate-buffered saline. Cells in phosphate-buffered saline were treated in order to stain the DNA using the DNAPrep Coulter Reagent Kit (Beckman Coulter, Brea, CA, USA), as specified by the supplier. Samples were subjected to fluorescence-activated cell sorting flow cytometry in a Coulter Epics Elite (Beckman Coulter). Cell-cycle analysis, focused on the G_2M phase in which malignant cells are arrested by taxanes, was performed 1, 3 and 7 days after each treatment.

Statistical analysis. For differences in cell proliferation between the various experimental treatments, *t*-test analysis with GraphPad Prism 7 Software (San Diego, CA, USA) was performed, while significance of differences in the proportion of cells in the G_2-M phase after the various experimental treatments were determined with chi square analysis with GraphPad Prism 7 Software. Differences with a *p*-value of less than 0.05 were considered significant.

Results

Inhibition of cell proliferation with different drug concentrations. Under normothermic conditions, higher concentrations of either paclitaxel or docetaxel overall resulted in significantly reduced cell proliferation in both cell lines ($p<0.05$, Figures 1 and 2). No difference in cytoxicity was observed after 7 days between results under the highest concentrations of docetaxel in both cell lines, most probably because further decrease of an already very low cell proliferation rate would not be feasible.

At 37˚C docetaxel appeared to be significantly ($p<0.05$) more effective than paclitaxel in reducing cell proliferation of SKOV-3 cells at all drug concentrations and time points and in OVCAR-3 cells at lower concentrations of 0.1 and 1 μM (Figures 1 and 2).

Hyperthermia versus normothermia. Cell proliferation: When comparing the effect of different concentrations of paclitaxel on SKOV-3 cell proliferation under normothermic and hyperthermic conditions (Figure 1), temporary thermal enhancement of the drug effect was observed only for 0.1 μM (cell proliferation rate: 99.8% *vs*. 88.0%, $p=0.001$) and 1 μM paclitaxel (99.4% *vs*. 87.3%, $p<0.0001$) at 24 h. At 7 days, no difference in cell proliferation was shown. Data from cell proliferation of OVCAR-3 cells were slightly different when cells were treated with 1 μM paclitaxel; a higher cell proliferation rate was observed in hyperthermia-treated cells at 24 h (98.5% *vs*. 85.3%, $p=0.014$), whereas thermal enhancement of paclitaxel effect was shown at 7 days (37.7% *vs*. 23.5%, $p=0.0023$). There were no significant differences for other concentrations at the different time points.

In SKOV-3 cells hyperthermia enhanced the effect of docetaxel at 0.1 μM (92.1% *vs*. 80.8%, $p=0.0039$) and 1 μM (93.0% *vs*. 79.7%, $p=0.0004$) after 24 h, but not at 7 days

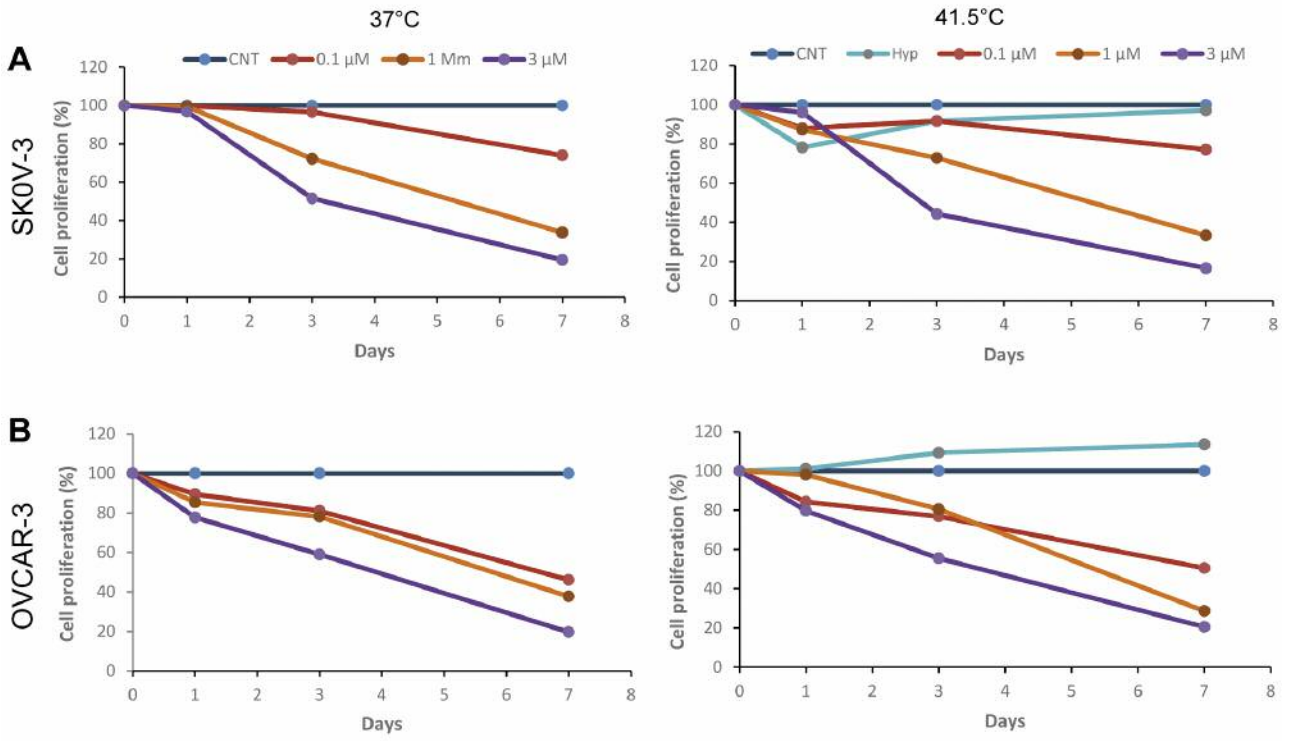

Figure 1. Figure 1. *Effect of 2-hour treatment with different concentrations (0.1, 1 and 3 µM) of paclitaxel under normothermic (left panel) and hyperthermic (Hyp; right panel) conditions on the proliferation of SKOV-3 (A) and OVCAR-3 (B) cells relative to that of the non-treated cell population (CNT). The treated and untreated cells were cultured under normal conditions for 1, 3 and 7 days. Cell proliferation was measured using MTT assay. For statistical significance of differences among values please see the text.*

(Figure 2). A very similar cell proliferation-inhibitory effect was shown in cells treated only with hyperthermia. In OVCAR-3 cells, there were no statistically significant differences between the effect of docetaxel under normothermic *versus* hyperthermic conditions.

Notably, 2-h hyperthermia without drug administration resulted in an increase, although statistically non-significant, of the OVCAR-3 cell proliferation rate at 3 and 7 days, while such a phenomenon was not observed in SKOV-3 cells.

Cell-cycle analysis: Cell-cycle analysis showed a differential effect of treatments on the G_2/M subpopulation in the two cell lines used (Figure 3).

Seven days after exposure of SKOV-3 cells to 1 µM paclitaxel, we found a significant increase of cells in the G_2/M-phase at hyperthermic comparing to normothermic conditions (14.6% *vs*. 0.7%, p=0.0003). Such a difference was not observed for 1 µM docetaxel. Treatment of OVCAR-3 cells with either 1 µM paclitaxel or docetaxel under hyperthermic conditions resulted in a significantly higher proportion of cells in the G_2/M-phase at 7 days (12.6% *vs*. 0%, p=0.0002 and 34.9% *vs*. 2.6%, p<0.0001, respectively). Notably, exposure to 1 µM docetaxel under hyperthermic conditions let to a significantly higher percentage of OVCAR-3 cells in the G_2/M-phase at 7 days in comparison to paclitaxel under the same conditions (p=0.0004).

Exposure of SKOV-3 cells to both paclitaxel, and docetaxel at 3 µM under hyperthermic conditions resulted in a significantly higher proportion of cells in the G_2/M-phase at 7 days (15.2% *vs*. 3.3%, p=0.0003 and 15.2% *vs*. 1.2%, p=0.005, respectively). Similar data were obtained for OVCAR-3 cells (38.6% *vs*. 0%, p<0, 0001 and 17.7% *vs*. 5.1%, p=0.015, respectively). Contrarily to the case of 1 µM concentration, exposure to 3 µM paclitaxel under hyperthermic conditions let to a significantly higher percentage of OVCAR-3 cells in the G_2/M-phase at 7 days than did exposure to docetaxel (p=0.0016).

Overall, our results show that, in general, hyperthermia when combined with drug treatment caused a delay in the G_2/M phase in comparison to treated cells without hyperthermia, although the percentage of cells in G_2/M was very close to that of untreated cells in both cell lines. Interestingly, when OVCAR-3 cells were treated only with hyperthermia, an opposite effect, showing a decrease of the G_2/M sub-population, was observed at 7 days (p<0.0001).

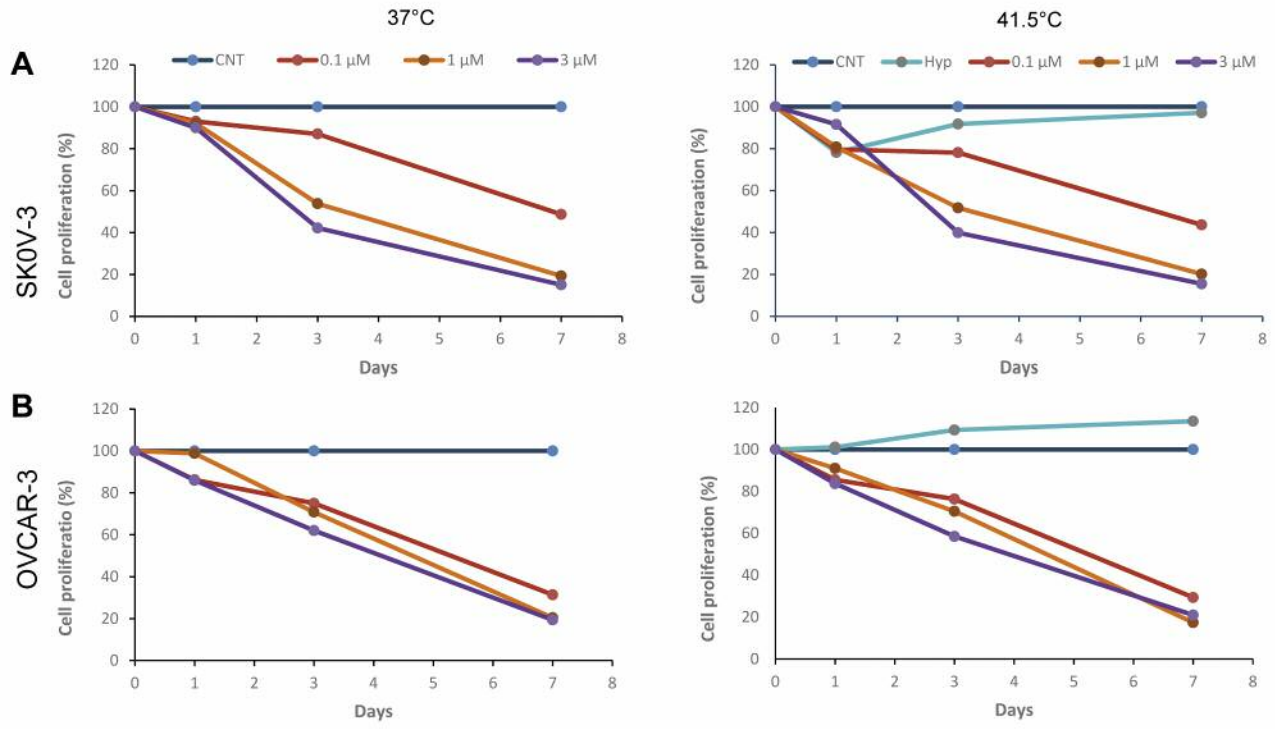

Figure 2. *Effect of 2-hour treatment with different concentrations (0.1, 1 and 3 μM) of docetaxel under normothermic (left panel) and hyperthermic (Hyp; right panel) conditions on the proliferation of SKOV-3 (A) and OVCAR-3 (B) cells relative to that of the non-treated cell population (CNT). The treated and untreated cells were cultured under normal conditions for 1, 3 and 7 days. Cell proliferation was measured using MTT assay. For statistical significance of differences among values please see the text.*

Discussion

The slow absorption of intraperitoneally delivered paclitaxel and docetaxel from the abdominal cavity, mainly due to their high molecular weight and hydrophobic properties, and their first-pass hepatic metabolism, results in high intraperitoneal drug concentrations and exposure of intraperitoneal tumor cells to these drugs, with simultaneously low systemic drug concentrations and hence limited systemic toxicity (3, 4, 19-21). This pharmacokinetic advantage is reflected by the ratio of the areas under the curve for drug concentration *versus* time for intraperitoneal and intravenous application of 550-2,300 and 150-3,000 for paclitaxel and docetaxel, respectively, and only 15-22 for platinum compounds (3, 4, 19, 20). As demonstrated in the present and other studies (22), higher paclitaxel and docetaxel concentrations are associated with increased inhibition of cell proliferation. Short-term exposure of cancer cells to high concentrations of taxanes appears effectively to induce long-term inhibition of cell proliferation and cell death. Considering their highly favorable pharmacokinetic profile, their high efficacy against ovarian cancer and the correlation of exposure to treatment response (1, 2), paclitaxel and docetaxel are attractive agents for intraperitoneal chemotherapy in patients with advanced ovarian cancer. The GOG172 randomized trial demonstrated a highly significant survival benefit for the combined postoperative regimen of intraperitoneal chemotherapy including paclitaxel and intravenous chemotherapy *versus* postoperative intravenous chemotherapy alone after CRS for advanced ovarian cancer (5). Consequently, its postoperative intraperitoneal administration is considered beneficial in the current National Comprehensive Cancer Network guidelines (6). Docetaxel may even be more effective, since in the present study, docetaxel was more cytotoxic than paclitaxel at similar concentrations. In a randomized trial, CRS and HIPEC with cisplatin and paclitaxel appeared superior to CRS alone for recurrent ovarian cancer (23). Other clinical studies also demonstrated encouraging results of HIPEC including paclitaxel or docetaxel for advanced ovarian cancer (19, 24-32), while currently both are being used for HIPEC in ongoing randomized trials (NCT02681432, NCT03373058 and NCT04280185) (33).

As mentioned earlier, hyperthermia may be directly cytotoxic and enhance the cytotoxic effect and tissue

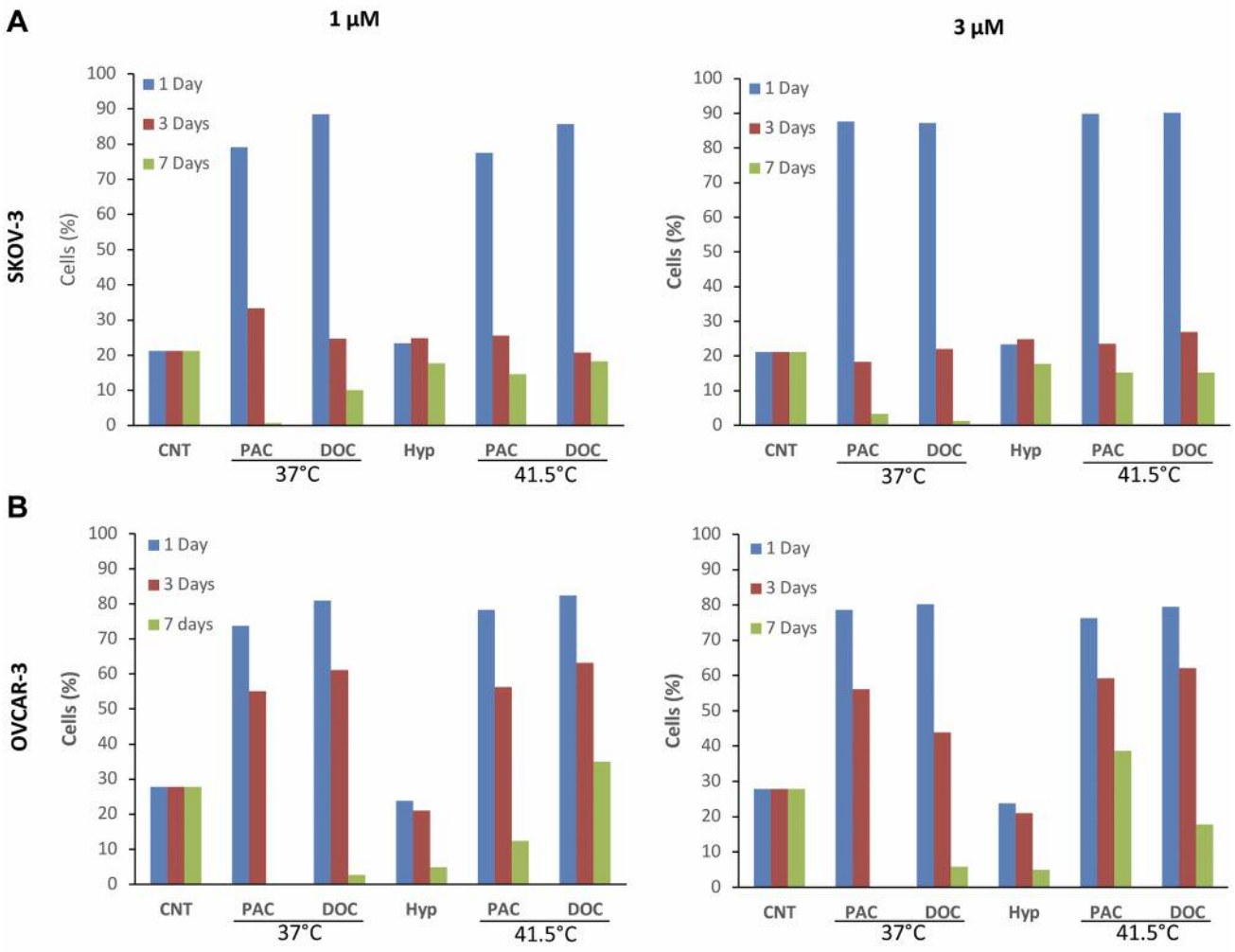

Figure 3. *Effect of different treatments on the G_2/M subpopulation of SKOV-3 (A) and OVCAR-3 (B) cells. Cells were treated with 1 and 3 µM paclitaxel (PAC) or docetaxel (DOC) at 37˚C and 41.5˚C for 2 h and then cultured under normal conditions for 24 h, 3 days and 7 days. The total number of cells were analyzed by fluorescence-activated sorting and the percentage of cells in the G_2/M phase is presented. For statistical significance of differences among values please see the text.*

penetration of chemotherapeutics (4, 7, 10-12). Moreover, hyperthermia can elicit antitumor immune responses by various mechanisms (34). Despite its potential beneficial cytotoxic effects, the addition of hyperthermia to intraperitoneal chemotherapy may have a negative influence on oncological outcome. In a mouse melanoma model, hyperthermia seemed to cause immunosuppressive effects and subsequently disease progression (35). Moreover, the enhanced systemic release of heat-shock proteins (HSPs) during HIPEC may result in reduced response to hyperthermia as well as chemotherapy (36-40). Additionally, HSPs have anti-apoptotic properties and promote tumor cell proliferation, invasiveness and metastatic dissemination (39, 41). In an experimental study, paclitaxel totally inhibited the expression of HSP27 in BG-1 ovarian cancer cells (42), suggesting that paclitaxel may neutralize the tumor-promoting effects of hyperthermia. In the present *in vitro* study, 2-h hyperthermia alone resulted in a decrease of the G_2/M subpopulation and although statistically non-significant, an increased proliferation rate of OVCAR-3 cells after 3 and 7 days, whereas concurrent administration of paclitaxel and docetaxel seemed generally to abrogate this adverse effect. Although interleukin-6 stimulates immune-mediated tumor control, hyperthermia-induced interleukin-6 release may also promote tumor cell proliferation, survival, angiogenesis, as well as evasion of immune surveillance in the tumor microenvironment (43). In an *in vitro* study, colon cancer cells that were incubated with 5-fluoruracil or mitomycin-C under hyperthermic condition showed increased cell viability

compared with normothermic incubation with these agents (39). Using tissue samples from peritoneal metastases of colon cancer before and after HIPEC, cancer cells that were able to survive the cytotoxicity of hyperthermia and chemotherapy demonstrated increased cell viability and proliferation (39).

An additional disadvantage of the use of hyperthermia is the risk of additional morbidity because heat can be harmful to normal tissues and intra-abdominal organs (13). CRS and HIPEC for primary and secondary peritoneal malignancies is associated with significant morbidity, which is often considered to be mainly attributed to the major surgery rather than HIPEC (44). Anastomotic leakage, especially in combination with chemotherapy-induced neutropenia, contributes considerably to its morbidity. In most experimental studies (45-50), HIPEC resulted in impaired healing of intestinal anastomoses. Although this may be primarily due to the intraoperative intraperitoneal chemotherapy rather than the hyperthermia (51), the contribution of hyperthermia may still be significant. In some animal models, local hyperthermia was associated with impaired anastomotic healing (46, 49) and in other experimental studies not (45, 50, 52, 53). In a recent study on a rat model, normothermic intraperitoneal chemotherapy with paclitaxel or hyperthermic perfusion with normal saline solution had no negative impact on anastomotic healing but the combination of hyperthermia and intraperitoneal chemotherapy with paclitaxel significantly reduced the mechanical strength of colonic anastomoses (50). Moreover, hyperthermia causes dysfunction of the tight junctions that, together with a single layer of enterocytes, form the intestinal epithelial barrier, resulting in augmented permeation of luminal antigens, endotoxins, and bacteria into the bloodstream (54). Additionally, hyperthermia during HIPEC causes a generalized inflammatory response, as evidenced by rising levels of, among other mediators, interleukin-6 and procalcitonin, resulting in severe hemodynamic derangements (55). This response to heat mimics conditions as observed during sepsis and septic shock and may contribute to increased operative morbidity and mortality. In a propensity score-matched study, HIPEC when compared with normothermic intraoperative intraperitoneal chemotherapy (NIPEC) with oxaliplatin after CRS in 90 colorectal cancer patients was not associated with statistically significantly increased morbidity and mortality, although trends of increased major complications, cardiovascular complications, infections in general and wound infections were observed (56). Additionally, hyperthermia may increase the drug uptake from the abdominal cavity and cause increased systemic toxicity (56).

A drawback of the addition of hyperthermia to intraperitoneal chemotherapy may be the fact that it has been introduced empirically, without having been the subject of clinical trials and without knowing the ideal target temperature. In a recent, not finally published, small randomized study (NCT02739698) (33), complete pathological response of post-treatment biopsies was not significantly different after HIPEC *versus* NIPEC with paclitaxel in patients with ovarian cancer. Studies comparing survival outcome of NIPEC with HIPEC with paclitaxel or docetaxel after CRS have not been published to date. In the single published randomized study that compared these treatment modalities, only a subgroup of patients with gastric cancer, serosal invasion and absence of visible peritoneal carcinomatosis exhibited a statistically significant survival benefit after adjuvant HIPEC when compared with NIPEC with mitomycin C and cisplatin (57). Results of an ongoing randomized trial (NCT02567253) comparing HIPEC with NIPEC with cisplatin after CRS for advanced ovarian cancer are eagerly awaited (33). In an experimental animal study, rats with peritoneal carcinomatosis from colorectal origin treated by CRS lived longer after NIPEC than HIPEC with mitomycin-C. Weight loss during the first postoperative days as a marker of treatment-related toxicity was most pronounced in the HIPEC group, while rats which underwent abdominal perfusion with heated normal saline and CRS had significantly higher weight loss than those with CRS alone (58). These findings may suggest additional toxicity of hyperthermia. In recent studies on rats with intra-abdominal inoculation of ovarian cancer, which were however not subjected to CRS, NIPEC with cisplatin was associated with less morbidity and a small, statistically non-significant, increase of survival when compared with HIPEC with cisplatin (59, 60).

In the absence of similar studies for taxanes, we have to rely on experimental *in vivo* and *in vitro* studies of their thermal enhancement. Their association with hyperthermia is interesting because tubulin/microtubule complex seems to be temperature-dependent (61). Moreover, hyperthermia resulted in an increase of the microtubule polymer mass in toads (62), and in spontaneous tubulin polymerization in sea urchin eggs (63). As already mentioned, paclitaxel may neutralize the eventual tumor-promoting effects of hyperthermia by total inhibition of HSP27 expression in ovarian cancer (42). As shown in Tables I and II, the data on the phenomenon of thermal enhancement for paclitaxel and docetaxel are inconsistent. The experimental studies varied in degree, duration and timing of hyperthermia, drug concentration and exposure time, time of cytotoxicity measurement after treatment, and cell type studied. In two studies, a statistically significant inhibition of drug cytotoxicity was even observed when 1-h hyperthermia was concurrently added at the beginning or the middle of 24-h exposure to paclitaxel (64, 65). In spite of causing a higher concentration of paclitaxel to be present in heated breast cancer cells, hyperthermia seemed to inhibit paclitaxel-related cell-cycle effects (65). A recent study demonstrated that the synergistic or even inhibition effect on breast cancer cells highly depends on paclitaxel dose and degree of hyperthermia (66). Similarly, in the present study, the existence of any thermal enhancement often depended on drug

Table I. *Experimental studies on the impact of concurrent hyperthermia on paclitaxel activity.*

Study	Year	Concentration/ dose paclitaxel	Exposure to paclitaxel	Temp., ˚C	Hyperthermia duration, min	Timing of hyperthermia[#]	Cell type	Effect assessed	Thermal enhancement
In vitro									
Rietbroek *et al*. (64)	1997	1-12 μM	24 h	41.8	60	First hour	Rat glial tumor cells (L-929)	7 Days	No
Rietbroek *et al*. (64)	1997	0.0001-10 μM	1, 4, 24 h	43	60	First or last hour[##]	Human squamous lung cancer (SW1573) and murine rhabdomyo-sarcoma cells (R1)	8-12 Days	No
Leal *et al*. (65)	1999	5, 10, 100 nM	24 h	43	60	Start, middle or end	Human breast adenocarcinoma cells (MCF-7)	26 H	No
van Bree *et al*. (71)	2000	2, 10, 30, 100 nM	24 h	41	60	Last hour	Various human colorectal cancer cell lines	10-12 Days	No
Othman *et al*. (72)	2001	10 μM	2 h	43	60	Last hour	Murine breast cancer cells (FM3A)	1, 2 and 24 H	Yes
Michalakis *et al*. (73)	2005	10-20 μM	2 h	41.5, 43	120	Entire paclitaxel exposure	Human cervical cancer cells (HeLa)	3 and 7 Days	No
Michalakis *et al*. (79)	2007	10-20 μM	2 h	41.5, 43	120	Entire paclitaxel exposure	Human mammary (MCF-7), ovarian (SKOV-3) and liver cancer (HEpG2) cells	3 and 7 Days	Yes/No*
Muller *et al*. (22)	2012	0.11-0.88 μM	30, 60, 90 min	38-43	30, 60, 90	Entire paclitaxel exposure	Human ovarian cancer cells (SHIN-3)	10 Days	No
Lin *et al*. (69)	2013	2-100 nM	24 h	41	2×120	At start and after 24 h	Human breast cancer cells (MCF-7)	12, 24, 36, 48 H	Yes**
Liao *et al*. (66)	2019	5 or 10 μg/ml	2 h	40, 40.5, 41	120	Entire paclitaxel exposure	Human breast cancer cells (MCF-7)	24 and 48 H	Yes***
Present study	2020	0.1, 1 or 3 μM	2 h	41.5	120	Entire paclitaxel exposure	Human ovarian (SKOV-3 and OVCAR-3) cancer cells	1, 3, 7 Days	No****
In vivo									
Cividalli *et al*. (74, 75)	1999	30, 45 mg/kg	*i.p.* injection	41, 42, 43	60	Various, before and after	Murine mammary adenocarcinoma transplanted in foot of mice	Clinical follow-up	Yes
Mohamed *et al*. (76)	2003	75, 150 mg/m^2	*i.p.* injection	41.5	30	Just after *i.p.* injection	Murine fibro-sarcoma cells (FSa-II) injected in foot of mice	Clinical follow-up	No
Boissenot *et al*. (77)	2017	5 mg/kg	*i.v.* injection	41-43	10	Day 1, 3 and 7	CT-26 colon cancer injected in flank of mice	Follow-up 13 days	Yes

Temp.: Temperature; *i.p.*: intraperitoneal; *i.v.*: intravenous. [#]Relative to exposure to paclitaxel; [##]and 24 or 48 h after exposure to paclitaxel; [###]1 h hyperthermia at beginning, middle or end of treatment with paclitaxel; [####]at last hour of exposure to paclitaxel; *only at 43˚C in MCF-7, not in SKOV-3 cell line; **only for 10-25 nM after 48 h; ***however, 5 μg/ml at 40˚C resulted in enhanced cell proliferation when compared with 37˚C; ****at 7 days, except for 1 μM in OVCAR-3 cell line.

Table II. *Experimental studies on the impact of concurrent hyperthermia on docetaxel activity.*

Study	Year	Concentration/ dose docetaxel	Exposure to docetaxel	Temp., ˚C	Hyperthermia#		Cell type	Assessment of effect	Thermal enhancement
					Duration, min	Timing			
In vitro									
Rietbroek *et al*. (64)	1997	0.1-1.2 μM	24 h	41.8	60	First hour	Rat glial tumor cells (L-929)	7 Days	No
Dumontet *et al*. (68)	1998	50 nM	60 min	43	60	Entire docetaxel exposure	Human erythro-leukemia (K562) and sarcoma cells (MESSA)	3 Days	No
Istomin *et al*. (78)	2008	10-25 ng/ml	60 min	42	60	Entire docetaxel exposure	Human cervical cancer cells (HeLa)	2 Days	No
Muller *et al*. (22)	2012	0.78-200 nM	30, 60, 90 min	38-43	30, 60, 90	Entire paclitaxel exposure	Human ovarian cancer cells (SHIN-3)	10 Days	No*
Wu *et al*. (70)	2017	52 nM	24 h	42	20	First 20 min	Human prostate cancer cells (PC-3)	3 Days	Yes
Present study	2020	0.1, 1 or 3 μM	2 h	41.5	120	Entire paclitaxel exposure	Human ovarian cancer (SKOV-3 and OVCAR-3) cells	1, 3, 7 Days	No
In vivo									
Mohamed *et al*. (76)	2003	175 and 350 mg/m^2	*i.p.* injection	41.5	30	Just after *i.p.* injection	Murine fibrosarcoma cells (FSa-II) injected in the foot of mice	Clinical follow-up	Yes
Mohamed *et al*. (67)	2004	350 mg/m^2	*i.p.* injection	41.5, 43.5	30, 90	After and/or 3 h after injection	Murine fibrosarcoma cells (FSa-II) injected in the foot of mice	Clinical follow-up	Yes/No**
Wu *et al*. (70)	2017	75 mg/m^2	Intratumoral injection	42	20	Just after drug injection	Human prostate cancer cells (PC-3) injected subcutaneously in the back of mice	MRI at 7 and 14 days	Yes

Temp.: Temperature; *i.p.*: intraperitoneaI. #Relative to exposure to docetaxel; *except for a 30-min treatment with docetaxel; **only for 90 min hyperthermia at 41.5˚C immediately after *i.p.* injection of paclitaxel.

concentration. Moreover, the timing of heat and its sequencing with drug exposure has been shown to be important for the existence of any synergistic effect (67, 68). Furthermore, the timing of treatment assessment is of importance. As shown in our study, an eventual initial inhibitory or synergistic effect may have disappeared 7 days after treatment. Hence, it might be that the synergistic effect of hyperthermia and paclitaxel (66, 69) or docetaxel (70) as assessed after 2-3 days in some *in vitro* studies would have been absent if the effect had been evaluated later, as in our study. Most reported *in vitro* as well as all *in vivo* experimental studies simulated treatment with intravenous chemotherapy and local hyperthermia delivered to the area of the tumor by external devices, *i.e.* long drug exposure and hyperthermia during a short period during or after drug exposure to tumor cells, or used cells of tumors that do not cause peritoneal carcinomatosis (Tables I and II) (64-78). Only in the report of Muller *et al*. (22) and a previously published study of our center (79), were conditions encountered during HIPEC protocol mimicked, with high drug concentrations, hyperthermia for 30 to 120 minutes, and tumor cell types that do cause peritoneal carcinomatosis (*i.e.* ovarian cancer cells). Muller *et al*. did not observe thermal enhancement for both paclitaxel and docetaxel in SHIN-3 human ovarian cancer cells, except for a 30-minute treatment with docetaxel. However, 30-minute hyperthermic treatment with docetaxel remained significantly less effective than 60-minute treatment with docetaxel at 37˚C (22). In a previous study of our center, significant thermal enhancement was not observed for high concentrations of paclitaxel at 41.5 and 43˚C for 2 h in SKOV-3 ovarian cancer cells (79). There are, however, some

significant differences between the present and the previously reported study. Firstly, both paclitaxel and docetaxel were used in two different ovarian cancer cell lines (SKOV-3 and OVCAR-3). Furthermore, paclitaxel concentrations were lower than in the previous study, but still high as in HIPEC, to provide more opportunity for additive cytotoxicity of hyperthermia. Since an intra-abdominal temperature of 43˚C is not usually reached at our center, hyperthermia treatment was performed only at 41.5˚C. In the present study, although a synergistic effect of hyperthermia and paclitaxel or docetaxel was observed after 24 h in some experiments, this impact did not persist until 7 days after treatment, despite the observation that hyperthermia appeared to result in an increased proportion of cells arrested in the G_2M-phase for a prolonged time. After 7 days, moderate thermal enhancement was only seen with 1 μM paclitaxel for OVCAR-3 cells. In a previous study, it was demonstrated that the inhibitory effect on cell proliferation by short exposure to high concentrations of paclitaxel was due both to cell-cycle arrest and cell death. Cell-death mechanisms seemed to be different under hyperthermic compared with normothermic conditions, although necrosis prevailed over apoptosis in both conditions (79).

Conclusion

The observed *in vitro* concentration-dependent cytotoxicity of both paclitaxel and, even more effective, docetaxel to ovarian cancer cells support their intraperitoneal use in the treatment of ovarian cancer. The lack of or limited thermal enhancement of their cytotoxicity in this experimental protocol mimicking conditions during HIPEC may suggest similar efficacy for NIPEC. Bearing in mind the above mentioned potentially oncological and treatment-related adverse effects of hyperthermia, further research to assess critically the necessity of hyperthermic conditions in this setting is warranted. Adequate comparative clinical studies will be difficult to perform. A more representative in-vitro model of peritoneal metastases of ovarian cancer with tumor-derived organoids (80, 81) may eventually contribute to determining the role of hyperthermia in intraoperative intraperitoneal chemotherapy with taxanes.

Conflicts of Interest

The Authors do not have any conflicts of interest to declare in relation to the study.

Authors' Contributions

EB and PT designed the study. DP, HP and ET performed all experiments. HP, DP and PT analyzed the results. EB, OZ and PT contributed to the interpretation of the results. EB wrote the article with contributions from DM, OZ and PT. All Authors have read and approved the final article.

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